The fabrication of supramolecular devices requires molecules that are capable of interlocking in a well-defined manner. Thus, molecular self-assembly systems that exploit the molecular-scale manufacturing precision of biological systems are prime candidates for supramolecular engineering. Although self-assembly of molecules is an ubiquitous strategy of morphogenesis in nature, researchers in the area of molecular nanotechnology and biomimetics are only beginning to exploit its potential for the functionalization of surfaces and interfaces as well as for the production of biomimetic membranes and encapsulation systems. This article presents an overview of a new approach in nanostructure technology and biomimetics based on crystalline bacterial cell surface layers.

Crystalline Bacterial Cell Surface Layers

Among the most commonly observed bacterial cell surface structures are monomolecular crystalline arrays of proteinaceous subunits termed S-layers [1], [2], [3]. S-layers have now been identified in hundreds of different species belonging to all phylogenetic groups of walled Eubacteria and represent an almost universal feature of archaeal cell envelopes (for compilation see [4]).
Morphological, chemical, genetic, and morphogenetic studies have shown that S-layers represent the simplest type of biological membrane developed during evolution. The crystalline arrays are composed of a single protein or glycoprotein species with molecular weights ranging from 40,000 to 200,000. S-layers exhibit either oblique (p1, p2), square (p4), or hexagonal (p3, p6) lattice symmetry with center-to-center spacings of the morphological units of 2.5 to 35 nm (Figure 1). Depending on the lattice type, morphological units are composed of one, two, three, four, or six identical subunits (Figure 2). S-layers are generally 5-10-nm thick and represent very porous meshworks with pores occupying 30-70% of their surface area. Pores in the protein lattice are of identical size and morphology in the 2-8 nm range. Often, two or even more distinct classes of pores can be detected.

Structural and chemical analysis of numerous S-layer lattices has demonstrated that they are highly anisotropic structures with regard to their inner and outer surface. In general, the outer surface is more hydrophobic than the inner one (for review see [5]-[8]). Chemical modification and immobilization experiments revealed a net negative surface charge for the inner face (due to an excess of carboxylic acid groups) and a charge neutral characteristic on the outer face (due to an equimolar amount of carboxylic acid and quaternary ammonium groups). Three-dimensional computer image reconstructions of the protein mass distribution [9], [10], ultrathin sections, and high-resolution metal shadowed freeze-dried preparations of S-layer self-assembly products [11] have shown that the outer surface of S-layer lattices is generally less corrugated than the inner one.

Based on the data obtained by fundamental studies on S-layers, a considerable potential for the crystalline arrays in biotechnology, biomedicine, biomimetics, and nonbiological applications, including areas such as molecular nanotechnology and microelectronics, became evident (for reviews see [3], [4], [12]-[18]).

Genetics

During the last years, numerous S-layer genes from organisms of quite different taxonomic affiliations have been cloned and sequenced [18]-[21]. Considering the competitive situation of closely related organisms in their natural habitats, it is obvious that the S-layer surface has to contribute to diversification rather than to conservation. S-layer variation was studied in detail for Campylobacter fetus, an important pathogen for humans and ungulates [22], [23] but was also observed for nonpathogens such as Geobacillus stearothermophilus [24]-[26]. Sequence identities among S-layer proteins are extremely rare. However, it is now evident that high sequence identities are limited to the N-terminal region that is responsible for anchoring the protein to the cell surface by binding to an accessory secondary cell wall polymer (SCWP) that is covalently linked to the peptidoglycan backbone. In this context, three repeats of S-layer homology (SLH) motifs, consisting of 50 to 60 amino acids each [27], have been identified at the N-terminal part of many S-layer proteins [20]. SLH motifs, if present, are involved in SCWP-mediated anchoring of the S-layer protein to the peptidoglycan layer [20], [28]-[35]. Studies on the chemical composition and structure of SCWPs from different organisms indicate a highly specific lectin-type recognition mechanism between the S-layer protein and the distinct type of SCWP [28]-[39]. S-layer proteins of gram-positive bacteria, like those of G. stearothermophilus wild-type strains [32], [40] and Lactobacillus [29], [41] do not possess SLH motifs. Nevertheless, the N-terminal part of G. stearothermophilus wild-type strains is highly conserved and recognizes a net negatively charged SCWP as the proper binding site [29], [32]. The production of different truncated forms of the S-layer protein SbsC of G. stearothermophilus ATCC 12980 confirmed that...
the N-terminal part is exclusively responsible for cell wall binding, but this positively charged segment is not involved in the self-assembly process [33] and seems to fold independently of the remaining protein sequence.

Structure-function relationship of distinct segments of different S-layer proteins has been elucidated in order to gain sufficient knowledge at which amino acid positions of the S-layer proteins foreign peptide sequences can be fused without disturbing the self-assembly and recrystallization process. For the S-layer protein SbpA of Bacillus sphaericus CCM 2177, it could be demonstrated that the C-terminally truncated form rSbpA31-1068 may be used as the base form for the construction of S-layer fusions proteins. Up to now, either the major birch pollen allergen Bet v1 (rSbpA31-1068/Bet v1) or a construction of S-layer fusions proteins. Up to now, either the major birch pollen allergen Bet v1 (rSbpA31-1068/Bet v1) or a camel antibody sequence recognizing lysozyme as an epitope (rSbpA31-1068/cAB) has been functionally incorporated [35], [42]. Owing to the versatile applications of the streptavidin-biotin interaction as a biomolecular coupling system, minimum-sized core-streptavidin (118 amino acids) was fused either to N-terminal positions of the S-layer protein SbsB isolated from G. stearothermophilus PV72/p2 or attached to the C-terminus of SbsB [43]. The fusion proteins and core-streptavidin were produced independently in Escherichia coli, isolated and refolded to heterotetramers consisting of one chain of fusion protein and three chains of streptavidin. The biotin binding capacity of the heterotetramers was ~80% in comparison to homotetrameric streptavidin, indicating that at least three of the four core streptavidin residues were accessible and active. Due to the capability of the heterotetramers to recrystallize in suspension, on liposomes, and on silicon wafers, this chimeric S-layer can be used as self-assembling nanopatterned molecular affinity matrix to arrange biotinylated compounds on a surface [18], [43].

Isolation and Self-Assembly

Most techniques for isolation and purification of S-layer proteins involve the mechanical disruption of the bacterial cells and subsequent differential centrifugation to separate the cell wall fragments. A complete solubilization of S-layers into their constituent subunits and their release from supporting layers can be achieved with high concentrations of hydrogen-bond breaking agents (e.g., guanidine hydrochloride), or by lowering or raising the pH. Summarizing the results from different disintegration procedures, it was concluded that i) in general eubacterial S-layer proteins are not covalently linked to each other or the supporting cell wall component, ii) differing combinations of weak bonds (hydrophobic, ionic, and hydrogen bonds) are responsible for the structural integrity of S-layers, and iii) bonds holding the S-layer subunits together are stronger than those binding S-layer lattices to the underlying cell envelope, which is seen as a basic requirement for continuous recrystallization of the crystalline array during cell growth [11], [44].

Self-Assembly in Suspension

S-layers isolated from a broad spectrum of prokaryotic organisms have shown the inherent ability to reassemble into two-dimensional arrays after removal of the disrupting agent used in the dissolution procedure (Figure 3) [11], [44]. The initial phase of this self-assembly process is determined by a rapid nucleation of protein subunits into assemblies of several unit cells. Subsequently, these aggregates reassemble in a much slower process into larger crystalline arrays. Most important for applied S-layer research, the formation of these self-assembled arrays is only determined by the amino acid sequence of the polypeptide chains and consequently the tertiary structure of the S-layer protein species [1], [45]. The self-assembly products may have the form of flat sheets, open-ended cylinders, or closed vesicles [11], [46], [47].

Reassembly of isolated S-layer proteins into larger crystalline arrays can be also induced on solid surfaces. In particular, the recrystallization of S-layer proteins on technologically relevant substrates such as silicon wafers, carbon-, platinum-, or gold electrodes and on synthetic polymers already revealed a broad application potential for the crystalline arrays in micro- and nanotechnology [3], [6], [48], [49]. The formation of coherent crystalline arrays strongly depends on the S-layer protein species, the environmental conditions of the bulk phase (e.g., temperature, pH, ion composition, and ionic strength) and, in particular, on the surface properties of the substrate. A broad screening program involving various surface properties revealed that hydrophobic surfaces are usually better suited for the formation of large-scale closed S-layer protein monolayers, although in some cases due to the anisotropic surfaces properties of S-layers recrystallization also occurs on hydrophilic substrates [6], [50].

High-resolution electron microscopical studies in combination with digital image processing have shown that crystal growth is initiated simultaneously at many randomly distributed nucleation points and proceeds in-plane until the crystalline domains meet, thus leading to a closed, coherent mosaic of individual several micrometer large S-layer domains [6], [50], [51].
Recrystallization at the Air/Water Interface and On Langmuir Lipid Films

Reassembly of isolated S-layer subunits at the air/water interface and on Langmuir-films has proven to be an easy and reproducible way for generating coherent S-layer lattices at large scales. In accordance with S-layers recrystallized on solid surfaces, the orientation of the protein arrays at liquid interfaces is determined by the anisotropy in the physicochemical properties of the protein lattice. Electron microscopical examinations revealed that recrystallized S-layers were oriented with their outer charge neutral, more hydrophobic face against the air/water interface and with their negatively charged, more hydrophilic inner face against the positively charged or zwitter-ionic headgroups of phospho- or tetraether lipid films [6], [52], [53]. As with S-layer lattices recrystallized on solid surfaces, S-layer protein monolayers consist of a closed mosaic of individual monocristalline domains.

The transfer of an S-layer protein monolayer or composite S-layer protein/lipid film onto a solid substrate may be performed by pushing the substrate vertically through the air/liquid interface (Langmuir Blockett technique; LB) or by depositing and lifting the substrate horizontally (Langmuir Schaefer technique; LS) from the surface. The combination of both methods allows a multiple transfer leading to multilayer assemblies.

Applications

S-layers have a broad spectrum of potential applications that are listed in Table 1 (for reviews see [6], [7], [12], [13], [16]-[18], [52]).

S-Layers as Matrix for Immobilizing Macromolecules

A controlled immobilization of molecules on surfaces is an essential requirement in most areas of supramolecular engineering and molecular nanotechnology. Contrary to conventional carriers where the location, local density, and orientation of functional groups and the porosity and pore size are only known approximately, with S-layer lattices, the properties of a single constituent unit are replicated with the periodicity of the lattice and thus define the characteristics of the whole two-dimensional array (for review see [5] and [6]). According to this principle it has already been shown that functional molecules can be bound to S-layers in dense crystalline packing.

Specific binding of molecules on S-layer lattices may be induced by different noncovalent forces. The pattern of bound molecules frequently reflects the lattice symmetry, the size of the morphological units, and the physicochemical properties on the array. For example, the distribution of net negatively charged domains on S-layers could be visualized by electron microscopical methods after labeling with positively charged topographical markers such as polycationic ferritin (PCF; diameter 12 nm) (Figure 4). The regular arrangement of free carboxylic acid groups on the hexagonal S-layer lattice from the archaea Thermoproteus tenax could be clearly demonstrated in this way [54].

Further on, S-layers have also shown to be particularly suitable as a matrix for a covalent attachment of functional macromolecules [55], [56]. For this purpose, carboxylic acid groups on

Table 1. Biomimetic and bio- and nanotechnological applications for S-layers (from [14]).

- Biomimetic S-layer ultrafiltration membranes (SUMs) with defined physico-chemical surface properties and molecular sieving characteristics
- SUMs, S-layer self-assembly products or S-layer microparticles as matrices for a controlled immobilization of functional molecules:
  - Covalent binding of enzymes for amperometric and optical bioanalytical sensors
  - Immobilized monoclonal antibodies for dipstick-style immunoassays
  - Immobilized protein A for escort-particles in affinity cross-flow filtration for isolation and purification of antibodies
  - Immobilized antibodies for preparation of microparticles for ELISA
- Supporting structures for functional lipid membranes at meso- and macroscopic scale:
  - “Semi-fluid” lipid membranes’ mimicking cell envelopes of Gram-negative archaea
- S-layer coated liposomes:
  - Immobilization of functional molecules on S-layer coated liposomes (e.g., addressor molecules for drug targeting)
  - Entrapping of functional molecules (drug delivery)
  - Artificial viruses
  - S-layer coated liposomes with immobilized antigens and haptens for vaccination
  - Conjugated vaccines: S-layers as carriers with intrinsic adjuvant properties for immobilization of antigens and haptens
  - Vehicle for producing fusion proteins: Homologous and heterologous expression of self-assembling fusion proteins (incorporation of functional domains as required for affinity matrices, enzyme membranes, vaccines, biosensors, and diagnostics)
- Biomimetic templates and nanonatural resist for semiconductor technologies
- Matrices for controlled biomimeralization
- Matrices for fabrication of metallic or semiconducting nanoparticles in nanoelectronics and optics

Fig. 4. (a) Polycationized ferritin (PCF) molecules are immobilized in a regular fashion by electrostatic interactions on the hexagonal S-layer of Thermoproteus tenax. The center-to-center spacing between the morphological units of the S-layer lattice and thus of the PCF molecules is 30 nm. (b) Ferritin is covalently bound in dense packing on the hexagonal S-layer of Thermoproteus tenax. Bars = 100 nm.
A controlled immobilization of molecules on surfaces is an essential requirement in most areas of supramolecular engineering and molecular nanotechnology.

the S-layer protein can be activated with carbodiimide while hydroxyl groups of the carbohydrate chains of S-layer glycoproteins are generally treated with cyanogen bromide or periodate. So far, S-layer lattices have been used as immobilization matrices for a broad spectrum of macromolecules of biologically active proteins (e.g., enzymes, antibodies, ligands) in the development of affinity membranes, biosensors, and medical diagnostics (for review, see [7], [12], [13], [56], and [57]).

In dipstick-style solid phase immunoassays, the respective monoclonal antibody was covalently bound to activated groups on the S-layer lattice [58]. Proof of principle was demonstrated for different types of S-layer ultrafiltration membranes (SUMs)-based dipsticks. SUMs are isoporous structures and were manufactured by depositing S-layer-carrying cell wall fragments under high pressure on commercial microfiltration membranes (MFMs) with an average pore size of approximately 0.4 µm [59], [60]. After deposition, the S-layer lattices are cross-linked to form a coherent smooth surface ideally suited for the attachment of functional macromolecules but also for depositing lipid membranes (see below). Dipstick-style immunoassays have been applied for diagnosis of type I allergies, for quantification of tissue type plasminogen activator (t-PA) either in patients’ whole blood or plasma, for monitoring t-PA levels in the course of thrombolytic therapies after myocardial infarcts, or for determination of interleukin 8 in supernatants of human umbilical vein endothelial cells (HUVEC) induced with lipopolysaccharides [18], [61], [62].

Alternative or complementary to existing S-layer technologies, genetic approaches are currently used for the construction of chimeric S-layer fusion proteins incorporating biologically active sequences without hindering the self-assembly of S-layer subunits into regular arrays on surfaces and in suspension. In the chimeric S-layer proteins rSbsC_{31,920}/Bet v1 and rSbpA_{41,1008}/Bet v1 carrying the major birch pollen allergen Bet v1 at the C-terminal end, the surface location and functionality of the fused allergen was demonstrated by binding Bet v1-specific immunoglobulin E (IgE) [35], [63]. These fusion proteins can be used for building up arrays for diagnostic test systems to determine the concentration of Bet v1-specific IgE in patients’ whole blood, plasma, or serum samples [62].

Based on S-layer technology a broad range of amperometric and optical bioanalytical sensors was developed at our research center (for review, see [56]). For example, for the fabrication of a glucose sensor, glucose oxidase molecules were covalently bound to the exposed S-layer surface of an SUM [64]. The electrical contact between the enzyme layer and the electrode was established by sputtering a thin layer of gold onto the sensing enzyme layer. The whole assembly was reinforced subsequently by attachment of a solid gold plate. The analyte reached the sensing layer through the open structure of the microfilter and the porous S-layer. The glucose concentration in the analyte was determined by measuring the current of the electrochemical oxidation of hydrogen peroxide produced in the course of the enzymatic reaction.

For the construction of multienzyme sensors, a different construction principle was developed where the individual enzymes were first immobilized to S-layer fragments that were subsequently mixed in suspension and finally deposited on a MFM [65]. The electrical contact was established by sputter coating as explained above. In this way, the immobilization parameters could be optimized for each enzyme individually and the ratio of the amounts of enzyme molecules accurately controlled. This method led to a well-structured sandwich of thin monomolecular enzyme layers where protective layers could easily be integrated.

On the basis of this technique several multienzyme sensors such as a sucrose sensor with three enzyme species (invertase-mutarotase-glucose oxidase) or a cholesterol sensor (with cholesterol esterase and cholesterol oxidase) were developed (for review, see [6]).

Further on, composite S-layer/enzyme layers were also used in the development of optical biosensors where the electrochemical transduction principle was replaced by an optical one [66]. In this approach, a pH- or oxygen-sensitive fluorescent dye was immobilized on the S-layer or brought into close proximity to the sensing layer. In the pH-sensitive system, with carboxyfluorescein as pH-sensitive dye, the glucose concentration in the sample was derived from the change in pH in the microenvironment between the dye- and the enzyme molecules. The pH-drop itself was caused by the enzymatic reaction due to the production of gluconic acid. For the oxygen-sensitive system a ruthenium(II)-complex was used as a transducer. The fluorescence of the ruthenium(II)-complex is dynamically quenched by molecular oxygen. Thus, a decrease in the local oxygen pressure can be detected as a result of the enzymatic reaction. S-layers were also used in the development of an infrared optical biosensor where an S-layer recrystallized on the cylindrical part of an infrared transparent optical fiber was used to bind glucose oxidase. The glucose concentration was determined from the infrared spectrum of gluconic acid [67].

S-Layers on Solid Supports

Among a broad spectrum of technical applications for S-layers recrystallized on solid substrates, the use of S-layer pro-
tein monolayers as “nanonatural resists” has already proven to be of practical importance [68], [69]. In this approach S-layer protein is recrystallized on a silicon or gallium arsenide wafer and patterned by exposure to deep ultraviolet (DUV) excimer laser radiation (ArF; wavelength = 193 nm) (Figure 5). The S-layer is removed specifically from the silicon surface but retains its crystalline and functional integrity in the unexposed areas. Subsequently, the remaining unexposed S-layer areas could be used either to bind enhancing ligands or to enable electroless metallization in order to form a layer that allows a final patterning process by oxygen-reactive ion etching. Since S-layers are only 5- to 10-nm thick and consequently much thinner than conventional resists, considerable improvement in edge resolution in the fabrication of submicron structures can be expected.

Recently it has been demonstrated that S-layers may also be used as the top layer on spin-coated polymeric resists [69]. Patterning of the two-layer resist system involved KrF radiation (wavelength = 248 nm) and ArF radiation. The larger wavelength is suitable for patterning the polymeric resist without altering the S-layer lattice. Consequently, the S-layer was structured first by ArF radiation and subsequently used as a mask for patterning the polymeric resist by blank exposure with KrF radiation. This two-step technique yielded very steep sidewalls in the polymeric resist material and subsequently in the silicon.

As an alternative to the application in the microelectronic sciences, unexposed S-layers may also be used for selectively binding intact cells (e.g., neurons) lipid layers, and/or biologically active molecules as required for the development of supramolecular structures or cell/semiconductor interfaces [70]-[73]. Although prototypes of such novel biosensors have not been developed yet, the fabrication would follow the procedures that have already been optimized for immobilizing macromolecules or for transferring lipid films on S-layer coated substrates.

**Biocrystal Templating (Biomineralization)**

Currently there is great interest in fabricating nanostructures for the development of a new generation of electronic and optic devices. In particular, the formation of metal clusters for nanoelectronic digital circuits requires well-defined sizes and arrangement of particles. As an alternative to approaches where colloidal crystallization was used to make close-packed nanoparticle arrays [74], the use of S-layers as organic templates allows the synthesis of a wide range of inorganic nanocrystal superlattices (for review, [6]). Recently, it was demonstrated that S-layer proteins recrystallized on solid supports or S-layer self-assembly products that were deposited
on such substrates may be used to induce the formation of CdS particles [75] or gold nanoparticles [76]. CdS inorganic superlattices with either oblique or square lattice symmetries of approximately 10-nm repeat distance were fabricated by exposing self-assembled S-layer lattices to Cd(II) solutions followed by slow reaction with hydrogen sulfide. Precipitation of the inorganic phase was confined to the pores of the S-layers with the result that CdS superlattices with prescribed symmetries were prepared [75].

In a similar procedure, a square superlattice of uniform 4- to 5-nm sized gold particles with 13.1-nm repeat distance was fabricated by exposing a square S-layer lattice, in which thiol groups had been introduced before, to a tetrachloroauric(III) acid solution (Figure 6). Transmission electron microscopical studies showed that the gold nanoparticles were formed in the pore region during electron irradiation of an initially grainy gold coating covering the whole S-layer lattice [76]. The shape of the gold particles resembled the morphology of the pore region of the square S-layer lattice. By electron diffraction and energy dispersive X-ray analysis, the crystallites were identified as gold (Au(0)). Electron diffraction patterns revealed that the gold nanoparticles were crystalline but in the long-range order not crystallographically aligned. These experiments were repeated with a broad range of different metal salts such as PdCl₂, NiSO₄, KPtCl₄, Pb(NO₃)₂, and K₃[Fe(CN)₆]. It should be stressed that with S-layers as molecular templates, the formation of superlattices with a wide range of interparticle spacings as well as with oblique, square, or hexagonal lattice symmetry becomes possible. This is particularly important for the development of nanometric electronic or optical devices since isolated S-layer subunits have shown the inherent capability to recrystallize on a great variety of solid supports including semiconductors (see above).

S-Layer Stabilized Lipid Membranes

The possibility for recrystallizing isolated S-layer subunits into large-scale isoporous, coherent protein lattices at the air/water interface or on lipid films and for handling such layers by standard LB techniques opened up a broad spectrum of applications in basic and applied membrane research including physiology, diagnostics, and biosensor developments (for review, see [3], [6], [7], [8], [17], [52], and [77]). S-layer supported lipid membranes strongly resemble those archael envelope structures that are exclusively composed of an S-layer and a closely associated plasma membrane. We have demonstrated that S-layer supported LB-films can cover holes or apertures up to several microns in diameter and maintain their structural and functional integrity in the course of subsequent handling procedures for a much longer period of time in comparison to unsupported structures (e.g., black lipid membranes) [3], [51], [78]. The stabilizing function of S-layers is primarily explained by a reduction or inhibition of horizontal vibrations that are seen as the main cause for disintegration of planar lipid membranes (Figure 7). Particularly stable composite structures can be obtained after intra- and intermolecular cross-linking the S-layer proteins alone or with molecules from the lipid layer from the subphase (e.g., with glutaraldehyde). The terminology “semifluid membranes” has been used to describe S-layer-supported membranes since the interaction of the lipid head groups with the repetitive domains of the associated S-layer lattice significantly modulates the characteristics of the lipid film (particularly its fluidity and local order on the nanometer scale) [78]. Fluorescence recovery after photobleaching (FRAP) measurements demonstrated that the mobility of lipids in S-layer-supported bilayers was higher than in other model systems, such as hybrid bilayers or dextran-supported bilayers [79]. Additional lipid layers or S-layer supported lipid layers can be deposited on such semifluid membranes by standard LB techniques or by fusion of lipid vesicles. Into S-layer stabilized lipid layers functional molecules may be incorporated using well-established procedures. The most promising candidates for such studies are functional molecules such as carriers, ion channels, proton pumps, photosynthetic reaction centers, light harvesting, and receptor molecules [6].

Fig. 6. Electron micrograph of gold nanoparticles on an S-layer with square lattice symmetry. Bar = 50 nm.

Fig. 7. Schematic representation of the “Semifluid membrane model.”
Recently, the electrophysical features of a composite S-layer/tetraetherlipid monolayer were investigated in comparison to unsupported tetraether lipid monolayers [80]. Tetraetherlipid monolayers were clamped with the tip of a glass pipette [Figure 8(a)]. This voltage clamp study indicated the formation of a tight lipid monolayer sealing the tip of the pipette, and a decrease in conductance of the lipid monolayer upon recrystallization of the S-layer protein. This clearly demonstrated that the S-layer protein had not penetrated or ruptured the lipid monolayer. Further on, differences in the incorporation of valinomycin into the tetraether lipid membrane indicated that the S-layer modified the accessibility and/or the fluidity of the lipid film. In contrast to plain lipid monolayers, S-layer supported lipid membranes persisted over longer periods of time, indicating enhanced stability. In a further study, the self-assembly of the pore forming protein α-hemolysin into an S-layer supported lipid membrane was investigated by applying the bilayer lipid membrane (BLM) technique [Figure 8(b)] [81]. The assembly of α-hemolysin into the composite structure was slow compared to plain lipid membranes due to an altered fluidity of the bilayer. No assembly could be detected upon adding α-hemolysin monomers to the S-layer-faced side of the composite membrane. Thus, the molecular sieving properties of the S-layer lattice did not allow the free passage of the α-hemolysin monomers and hindered the formation of lytic pores. Again, in comparison to plain lipid bilayers, the S-layer supported lipid membrane had a decreased tendency to rupture. A recent study even demonstrated single pore recordings of α-hemolysin reconstituted in S-layer supported lipid membranes [82].

Lipid membranes attached to a porous support combine the advantage of possessing an essentially unlimited ionic reservoir on each side of the lipid membrane and of easy manual handling. A new strategy is the application of an SUM with the S-layer as stabilizing and biochemical layer between the BLM and the MFM. Composite SUM-supported bilayers are tight structures with breakdown voltages well above 500 mV during their whole lifetime of ~8 hours [83]. For a comparison, lipid membranes on a plain Nylon MFM revealed a lifetime of about three hours and ruptured at breakdown voltages of about 210 mV. Specific capacitance measurements and reconstitution experiments revealed functional lipid membranes on the SUM as the pore-forming protein α-hemolysin could be reconstituted to form lytic channels. For the first time, opening and closing behavior of even single α-hemolysin pores could be measured with membranes generated on a porous support [83]. The main phospholipid of Thermoplasma acidophilum (MPL), a membrane-spanning tetraether lipid, has also been transferred on an SUM by a modified LB-technique [84]. Again, SUM-supported MPL membranes allowed reconstitution of functional molecules, as proven by measurements on single gramicidin pores. Recrystallization of an additional monomolecular S-layer protein lattice on the lipid-faced side of SUM-supported MPL membranes increased the lifetime significantly to about one day [84].

Writing with Molecules on S-Layers

Corresponding to the classic work of Eigler and coworkers [85], who have demonstrated the possibility to write with atoms on solid metal surfaces, it should be possible to specifically manipulate macromolecules with the tip of a scanning force microscope (SFM; also known as an atomic force microscope) on the surface of S-layer lattices under aqueous environments. To prove this concept we have suggested to use as a model system PCF electrostatically bound in well-defined positions onto negatively charged domains on an S-layer lattice exhibiting large lattice spacings (Figure 9). The bound PCF molecules could be dragged with an electrically negatively charged (metal
coated) SFM tip and dropped at defined negatively charged domains on the S-layer lattice by reversing the polarity of the tip. Due to the crystalline arrangement of the negatively charged domains on the S-layer lattice, the deposited PCF molecules could generate geometric patterns resembling the periodicity of the underlying lattice. Theoretically, “writing with molecules” on S-layer lattices could also be done by selectively removing individual molecules from a lattice that in an initial step had been completely loaded with the respective molecules. Reading the information would only require to scan the native structure or a high-resolution replica of it with the SFM tip again. Theoretically, the storage capacity of such a device would be one terabit of binary information on 1 cm² for a square S-layer lattice with a lattice spacing of 10 nm. Currently, the most severe problems of such a new storage technology are seen in the necessity to store or read the information sequentially, in a quick erasure of the information and very critical environmental parameters for maintaining the integrity of a “biological storage device” over years.

Conclusions and Perspectives
This article has highlighted some of the biomimetic and nanotechnological applications of crystalline bacterial cell surface layers. As with most new basic technologies a considerable body of work will still be required to fully exploit the application potential of S-layers as nature-tailored supramolecular structures.

S-layers are the simplest self-assembly system leading to monomolecular isoporous protein lattices with repetitive physicochemical properties down to the nanometer scale. Being composed of identical protein or glycoprotein subunits, S-layers represent structures at the ultimate resolution limit for the molecular functionalization of surfaces and interfaces. For the first time, S-layer-stabilized artificial lipid membranes should enable the exploitation of functional principles of lipid membranes at meso- and even macroscopic scale. Incorporation of peptide stretches representing functional domains of other proteins by genetic engineering will broaden the application potential of S-layers and could lead to the development of new affinity and ion-selective binding matrices, enzyme membranes, biosensors, biocompatible surfaces or bioabsorbable systems [17], [18], [20]. Finally, S-layers appear to be particularly suitable as templates for controlled inorganic deposition. As already demonstrated, S-layer (glyco)proteins can be chemically modified with many different types of functional groups and molecules in fabricating a wide range of properties generating highly specialized matrices for wet chemical synthesis. Such strategies may allow fabrication of metal cluster arrays for applications in nanoelectronic circuits.

Fig. 9. Schematic illustration of writing with molecules on a hexagonal S-layer lattice. Molecules that are bound by electrostatic forces onto the S-layer may be transferred with the scanning force microscope (SFM) tip to a blank morphological unit in a controlled way. Depending on the prospective application, a negative (bottom left) or positive (bottom right) writing process can be applied.

Acknowledgments
Part of this work was supported by the Austrian Science Foundation (FWF) (Projects: P14419-MOB, P12938-MOB, and P16295-B07); the Volkswagen Foundation, Germany, Project
I77710; the Austrian Federal Ministry of Education, Science and Culture; and the Austrian Federal Ministry of Transport, Innovation, and Technology.

Uwe B. Sleytr received the Ph.D. in food and biotechnology at the University of Natural Resources and Applied Life Sciences (BOKU), Vienna, Austria, in 1968 and completed postdoctorate study at the Strangeways Research Laboratory and MRC Laboratory of Molecular Biology, Cambridge, U.K., in 1974-1975. Dr. Sleytr was a visiting professor at Temple University, Philadelphia, in 1977-1978. Since 1982 Dr. Sleytr has been a full professor and head of the Center for Ultrastructure Research and, since 1986, the director of the associated Ludwig Boltzmann Institute of Molecular Nanotechnology at BOKU in Vienna, Austria. Dr. Sleytr’s main research interests include molecular nanotechnology, nanobiotechnology, and biomimetics.

Bernhard Schuster received the Ph.D. in chemistry at the Graz University of Technology. He has been an assistant professor at the Center for Ultrastructure Research at the University of Natural Resources and Applied Life Sciences (BOKU) in Vienna, Austria, since 1995. His main research interests include nanobiotechnology and biomimetics and, in particular, functionalized lipid membranes.

Diethmar Pum received the Ph.D. in physics at the Vienna University of Technology in 1984. During his Ph.D. work, he spent two years at the Swiss Federal Institute of Technology in Zurich, Switzerland before starting as a university assistant at the Center for Ultrastructure Research at the University of Natural Resources and Applied Life Sciences (BOKU) in Vienna, Austria. Dr. Pum completed his postdoctoral work at the Arrhenius Laboratory, University of Stockholm, Sweden in 1987. Since 1997 he has been a university professor at BOKU. His main research interests are two-dimensional protein crystals, biomimetic membranes, and functionalized nanoparticles.

Address for Correspondence: Uwe B. Sleytr, Center for Ultrastructure Research and Ludwig Boltzmann-Institute for Molecular Nanotechnology, Universität für Bodenkultur Wien, Gregor Mendelstr. 33, A-1180 Vienna, Austria. Tel: +43 1 47654 2200. Fax: ++43 1 4789112. E-mail: uwe.sleytr@boku.ac.at. Web: http://www.boku.ac.at/zuf/.

References


